Recombinant wild-type measles virus containing a single N481Y substitution in its haemagglutinin cannot use receptor CD46 as efficiently as that having the haemagglutinin of the Edmonston laboratory strain

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Signalling lymphocyte activation molecule (SLAM) acts as a cellular receptor for Measles virus (MV). The recombinant MV, based on a SLAM-using clinical isolate in which asparagine at position 481 of the haemagglutinin was replaced with tyrosine, was generated. Characterization of this recombinant virus revealed that the N481Y substitution in the haemagglutinin allowed it to utilize CD46 as an alternative receptor, but that its ability to use CD46 was rather low in CD46+ cell lines compared with that of the recombinant virus possessing the haemagglutinin of the Edmonston laboratory strain. Thus, an N481Y substitution alone may not be sufficient to make SLAM-using MVs use CD46 efficiently, suggesting that further substitutions in the haemagglutinin are required for them to grow efficiently in CD46+ cells like the Edmonston strain. This may be a reason why few CD46-using MVs are detected in vivo.

Measles virus (MV), a member of the genus Morbillivirus in the family Paramyxoviridae, has two envelope glycoproteins, the haemagglutinin (H) and fusion (F) protein, which mediate receptor binding and membrane fusion, respectively (Griffin, 2001). CD46 acts as a cellular receptor for laboratory-adapted strains (Dörig et al., 1993; Naniche et al., 1993) as well as some clinical isolates of MV (Manchester et al., 2000). CD46 is a complement regulatory molecule and expressed on all nucleated cells in humans (Liszewski et al., 1991). On the other hand, MV strains isolated in the mar-moset B-cell line B95a or human B cell lines use signalling lymphocyte activation molecule (SLAM; also known as CD150), but not CD46, as a receptor (Erlenhöfer et al., 2001, 2002; Hsu et al., 2001; Tatsuo et al., 2000a, b). SLAM is expressed on certain types of cells of the immune system (Nichols et al., 2005) and shown to act as a receptor for all MV strains (Erlenhöfer et al., 2002; Yanagi et al., 2002). Indeed, the Edmonston laboratory strain uses both SLAM and CD46 as receptors (Tatsuo et al., 2000b). Other morbilliviruses such as Canine distemper virus and Rinderpest virus also use SLAM as a cellular receptor (Seki et al., 2003; Tatsuo et al., 2001).

The vast majority of CD46-using MV strains have tyrosine at position 481, whereas most B cell-isolated strains have an asparagine at that position. Studies have shown that a single asparagine to tyrosine substitution at position 481 (N481Y) enables the H proteins of B cell-isolated MV strains to interact with CD46, without compromising their ability to use SLAM (Bartz et al., 1996; Erlenhöfer et al., 2002; Hsu et al., 1998; Lecouturier et al., 1996; Nielsen et al., 2001; Shibahara et al., 1994; Xie et al., 1999). Furthermore, when SLAM-using strains were adapted to growth in the monkey kidney cell line, Vero, the N481Y substitution of the H protein was often observed after several passages (Nielsen et al., 2001; Schneider et al., 2002; Shibahara et al., 1994). In some Vero cell-adapted strains, a serine to glycine substitution at position 546 of the H protein was observed instead of the N481Y substitution (Li & Qi, 2002; Rima et al., 1997; Shibahara et al., 1994; Woelk et al., 2001).

We have previously shown that throat swabs from measles patients produced numerous plaques on SLAM-expressing Vero cells, but none (less than the detection limit) on Vero cells (Ono et al., 2001), indicating that most, if not all, MVs in the bodies of measles patients use SLAM but not CD46 as a receptor. Intuitively, the use of CD46 as a receptor appears to be more advantageous to MVs because distribution of CD46 is ubiquitous unlike that of SLAM. Furthermore, the adaptation to CD46 can occur easily in cultured cells by a single amino acid substitution at position 481 or 546 of the H protein. Then, why are few CD46-using viruses detected in vivo? To gain an insight into this problem, we generated the recombinant MV, based on a SLAM-using strain with a single N481Y substitution on the H protein. Such a virus should also emerge readily in vivo.

We have previously constructed the plasmid p(+ )MV323-EGFP encoding the full-length antigenomic cDNA of the IC-B strain (an isolate in B95a cells) with the enhanced green...
fluorescence protein (EGFP) gene and rescued the recombinant virus IC323-EGFP from it (Hashimoto et al., 2002; Takeda et al., 2000). We have also rescued IC323/EdH-EGFP, in which the H gene of the IC323-EGFP virus was replaced with that of the Edmonston strain (Hashimoto et al., 2002). We performed site-directed mutagenesis on p(+MV323-EGFP by using PCR (Vallejo et al., 1995), in which the N481Y substitution was introduced into the H protein and the recombinant virus IC323/H(N481Y)-EGFP was rescued, as described previously (Takeda et al., 2005).

Titres of virus stocks were determined by measuring p.f.u. on Vero/hSLAM cells, a Vero clone stably expressing human SLAM (Ono et al., 2001). The p.f.u. was comparable to infectious unit as determined by counting the number of EGFP-expressing cells.

In order to determine the receptor usage of the recombinant viruses, B95a (SLAM+ CD46−) and Vero (SLAM− CD46+) cells were pre-treated for 1 h with IPO3 (anti-human SLAM monoclonal antibody, 10 μg ml−1; Kamiya Biomedical) and M75 (anti-human CD46 monoclonal antibody, 10 μg ml−1) (Seya et al., 1990), respectively, and then infected in the presence of respective antibodies with 1×10^5 p.f.u. each of IC323-EGFP, IC323/H(N481Y)-EGFP or IC323/EdH-EGFP. EGFP autofluorescence was examined under a fluorescence microscope 24 h after infection (Fig. 1). All recombinant viruses produced syncytia in B95a cells treated with control mouse immunoglobulin G (IgG) (10 μg ml−1), but not in those treated with IPO3. On the other hand, IC323/H(N481Y)-EGFP and IC323/EdH-EGFP produced syncytia in Vero cells treated with control IgG, but not in those treated with M75. IC323-EGFP did not produce cytopathic effect in Vero cells treated with either control IgG or M75. Thus, the results show that IC323/H(N481Y)-EGFP can infect cells using CD46 as well as SLAM, like IC323/EdH-EGFP.

Next, we quantified entry efficiencies of the recombinant MVs on various cell lines (Fig. 2a). B95a and four CD46+ SLAM− cell lines (CHO/CD46, Vero, HeLa and Jurkat) (Tatsuo et al., 2000a) were infected with serially diluted virus solutions (the least diluted solutions of respective recombinant MVs were adjusted such that they would give 1×10^5 infectious units ml−1 on Vero/hSLAM cells). The fusion block peptide (Z-D-Phe-Phe-Gly) (Richardson et al., 1980) was added to the medium at a final concentration of 50 μg ml−1 after infection to prevent the second round of infection by progeny viruses. Infectious titres (reflecting virus entry, but not subsequent replication) were determined by counting the number of EGFP-expressing cells 24 h after infection. Titres were expressed in comparison to those on Vero/hSLAM cells (titre of each recombinant virus on Vero/hSLAM was set to 100 %). The three recombinant viruses showed similar entry efficiencies on B95a.

**Fig. 1.** Receptor usage of recombinant viruses. B95a cells were pre-treated with either control mouse IgG or IPO3, and Vero cells with either control mouse IgG or M75. The cells were infected with IC323-EGFP, IC323/H(N481Y)-EGFP or IC323/EdH-EGFP and then incubated with complete medium containing respective antibodies. EGFP autofluorescence was examined 24 h after infection.
cells. Infectious titres of IC323/EdH-EGFP on CHO/CD46, Vero, HeLa and Jurkat cells were 100 to 1000 times higher than the corresponding titres of IC323-EGFP, consistent with our previous study (Hashimoto et al., 2002). However, infectious titres of IC323/H(N481Y)-EGFP on these cell lines were only 3 to 30 times higher than those of IC323-EGFP. Accordingly, they were much lower than those of IC323/EdH-EGFP.

To confirm that the difference in the H protein (not indirect effects of mutations in other genes) is indeed responsible for the difference in entry efficiency, we used the vesicular stomatitis virus (VSV) pseudotype system in which the H protein of each recombinant virus was expressed together with the F protein of the IC-B strain on the surface of the virion, in the absence of the VSV G envelope protein (Takada et al., 1997; Tatsuo et al., 2000a). We also produced the pseudotype bearing the IC-B H protein containing an S546G substitution. B95a, CHO/CD46, Vero, HeLa and Jurkat cells were infected with serially diluted virus solutions (the least diluted solutions of respective pseudotypes were adjusted such that they would give $5 \times 10^4$ infectious units ml$^{-1}$ on Vero/hSLAM cells), and infectious titres were determined 24 h after infection (Fig. 2b). Titres were expressed in comparison to those on Vero/hSLAM cells (titre of each pseudotype on Vero/hSLAM was set to 100%). VSV pseudotype bearing the VSV G protein (VSVΔG*-G) and no envelope protein (VSVΔG*) were used as controls. Undiluted stock solution of VSVΔG* gave negligible titres on all cell lines tested (data not shown).

All five pseudotypes bearing envelope proteins exhibited similar titres on B95a cells. As expected, infectious titres of the VSV pseudotype bearing the H protein of the IC-B strain (VSVΔG*-ICHF) on CHO/CD46, Vero and HeLa cells were more than 2 logs lower than the corresponding titres of the pseudotype bearing the H protein of the Edmonston strain (VSVΔG*-EdHICF). Infectivity titres of the pseudotype bearing the IC-B H protein containing the N481Y substitution [VSVΔG*-ICH(N481Y)F] or that containing the S546G substitution [VSVΔG*-ICH(S546G)F] on CHO/CD46, Vero and HeLa cells were higher than the corresponding titres of VSVΔG*-ICHF, but much lower than those of VSVΔG*-EdHICF. The titres on Jurkat cells were somewhat different. Infectious titre of VSVΔG*-EdHICF on Jurkat cells was almost 2 logs lower than that of VSVΔG*-G, and VSVΔG*-ICH(N481Y)F and VSVΔG*-ICH(S546G)F showed almost the same low titre as VSVΔG*-ICHF. In general, entry efficiencies obtained with the VSV pseudotypes were consistent with those with the recombinant MVs. At present, we do not know why IC323/H(N481Y)-EGFP, VSVΔG*-ICH(N481Y)F and VSVΔG*-ICH(S546G)F hardly entered Jurkat cells. Infection of Jurkat cells with the Edmonston strain is mediated by CD46, as pre-treatment with M75 blocked it (data not shown). It has been reported that CD46 density determines entry efficiency by the Edmonston strain (Anderson et al., 2004), and we found by flow cytometry that Jurkat cells had lower mean fluorescence intensity (112±58) of the CD46 expression compared with other cell lines (CHO/CD46, 260±76; Vero, 188±03; HeLa, 211±16). Thus, the low-expression level of CD46 on Jurkat cells may at least partly explain why the viruses bearing the IC-B H protein containing the N481Y or S546G substitution entered Jurkat cells so inefficiently.

In order to determine how these differences in entry efficiency affect viral growth, we examined replication of the
recombinant viruses in Vero/hSLAM, HeLa and Jurkat cells (Fig. 3). The cell lines were infected with respective recombinant viruses at an m.o.i. of 0-1. Infected cells and medium were harvested at various time points after infection and virus titres were determined by measuring p.f.u. on Vero/hSLAM cells. All recombinant viruses replicated efficiently with similar kinetics in Vero/hSLAM cells. As expected, IC323/EdH-EGFP replicated well in HeLa and Jurkat cells, whereas IC323-EGFP hardly grew in them. IC323/H(N481Y)-EGFP replicated much more slowly in HeLa cells than IC323/EdH-EGFP, and its titres at 48 and 72 h after infection were roughly 2 logs lower than those of IC323/EdH-EGFP. IC323/H(N481Y)-EGFP did not replicate significantly in Jurkat cells. These results were in accordance with those obtained with virus entry.

In this study, we show that a single N481Y substitution in the H protein can indeed confer on a SLAM-using MV strain the ability to infect cells using CD46, but that its ability to use CD46 was much lower in CD46+ SLAM− cell lines compared with that of the recombinant virus possessing the H protein of the Edmonston strain. There are 17 predicted amino acid differences in the H protein between the Edmonston and IC-B strains (Takeuchi et al., 2002); although some of them are probably due to the genotype difference, others may be related to the ability of the Edmonston strain to use CD46 as a receptor. Most notable among the differences is Y481 found in the Edmonston strain. Upon substitution of this residue, the Edmonston strain is no longer able to infect cells using CD46 (Hsu et al., 1998; Lecouturier et al., 1996). On the other hand, SLAM− using MV strains come to utilize CD46 as a receptor when the residue at position 481 of the H protein is converted into tyrosine (Erlenhöfer et al., 2002; Hsu et al., 1998; Lecouturier et al., 1996; Xie et al., 1999; this study). However, Y481 may not be the only residue that enables the MV H protein to utilize CD46. Recent studies based on the structural modelling of the H protein suggest that many residues in two or three clusters are involved in the H protein’s interaction with CD46 (Massé et al., 2004; Vongpunsawad et al., 2004). Through mutations during passages on various types of cells (Parks et al., 2001), the Edmonston strain must have gradually increased the capacity to use CD46. Thus, the Edmonston strain is presumably able to use CD46 very efficiently. In fact, we have previously shown that IC323/EdH-EGFP enters SLAM- and CD46-expressing cells with similar efficiencies (Hashimoto et al., 2002), indicating that the Edmonston H protein can utilize SLAM and CD46 almost equally.

We have generated recombinant viruses in which part of the genome of the IC-B strain was replaced with the corresponding sequences of the Edmonston strain and found that the M and L genes of the Edmonston strain were also important for its efficient replication in Vero cells (Tahara et al., 2005). This may explain why the recombinant Edmonston virus bearing the WTF (an MV strain using SLAM only) H protein with an N481Y substitution grew as

![Fig. 3. Growth of recombinant viruses. Vero/hSLAM (a), HeLa (b) and Jurkat (c) cells were infected at an m.o.i. of 0-1 with IC323-EGFP (○), IC323/H(N481Y)-EGFP (■) or IC323/EdH-EGFP (■). The medium and cells were harvested at each time point and titres were determined on Vero/hSLAM cells.](https://www.microbiologyresearch.org/doi/10.1099/jgv.0.02220-0)
well as the Edmonston strain in Vero cells (Erlenhöfer et al., 2002) because the recombinant virus has the M and L genes from the Edmonston strain.

One proposed explanation for the lack of CD46-using MVs in vivo is that those viruses would downregulate CD46 from infected cells, which are then subject to complement-mediated lysis, and eliminated (Schnorr et al., 1995). Our present study provides another explanation. It indicates that a single N481Y (and probably S546G) substitution in the H protein alone cannot make a SLAM-using MV grow efficiently in CD46+ cells. The expansion of CD46-using MVs in vivo (and probably in vitro as well) may require further mutations in the H gene such that the mutated H protein can interact with CD46 more efficiently (like the Edmonston H gene) and/or other mutations that allow more efficient virus replication at post-entry steps (like the Edmonston M and L genes). Thus, CD46-using MVs may emerge and expand in culture where SLAM is not present, but they may not readily expand in vivo by competing with viruses using SLAM only.

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